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Cite as: AIP Conference Proceedings **1772**, 050007 (2016); <https://doi.org/10.1063/1.4964577>
Published Online: 13 October 2016

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Characterization of Chitin and Its Complexes Extracted from Natural Raw Sources

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Abstract. It is known that the main source of chitin and chitosan are shells of shrimp and other sea crustaceans. Alternative raw sources of chitin, chitosan and its complexes are the lowest plants – mushrooms and insects. Also industrial wastes, especially from brewing of beer and manufacture of wine and ethanol can be used for extracting chitosan-containing products. The present research is aimed to the extraction of chitin and its complexes from alternative raw sources, such as insects (cockroaches *Pariplaneta Americana linnaeus* and bees *Apis mellifera lineaus*), mushrooms (*Amanita phalloides* and *Lactarius subdulius*), waste banana wine (Kovibar and Urwibutso Inc.) and beer products (Bralirwa Inc., traditional sorghum) characteristic for Rwanda and their characterization using FTIR spectroscopy and elementary analysis. In chitin and its complexes extraction from all used raw sources, conditions for deproteinization were: 8% NaOH at 95 °C for 1 h and demineralization involved treatment with 6.7 % HCl at room temperature. Chitin and its complexes in the extracted samples were identified by FTIR spectroscopy using reference sample of *Aspegillus niger* mushrooms. The presence of chitin parts causes the absorption band at 1650, 1552 and 1376 cm⁻¹, which correspond to vibrations of amide groups «amide I», «amide II» and «amide III», respectively. Using elemental analysis, the ratios of chitin and glucan parts were estimated and the percentage of chitin composition of all species was determined. For most of raw sources a fraction of chitin part was greater than that of glucan part. The chitin content of the samples studied ranged between 0.7-0.8 % of DM (dried mass) for wine (beer) waste products and 38% of dried mass (DM) for cockroaches.

INTRODUCTION

Chitin and chitosan are ones of the most important key materials in the 21st century. They have multifunctional properties with applications in the pharmaceutical sciences, environmental problem-solving area (such as water-cleaning) to fibres, films and hydrogels, in cosmetics industry, food additives and as agricultural materials. Commercial chitosan is derived from the shells of shrimp and other sea crustaceans, including *Pandalus borealis* [1].

The active ingredient is also found in lower and higher fungi [2-4], silkworms [5,6], bees [7] and in certain other organisms. Given their low potential for toxicity and their abundance in the natural environment, chitin and chitosan are not expected to harm people, pets, wildlife, or the environment when used according to label directions [8].

Chitin, N-acetylated polymer of β -(1,4)-D-glucosamine, is the most important natural polysaccharide after cellulose found in nature. Its structure is identical to that for cellulose, however this compound contains acetylamido group at the second carbon atom of pyranose cycle instead of hydroxyl group characteristic for cellulose. Chitin can

be converted to chitosan by either homogeneous or heterogeneous alkaline *N*-deacetylation or by enzymatic means [8]. Chitosan possesses a large set of useful properties as compared to cellulose and its natural precursor chitin.

Chitin is insoluble in many solvents; it is difficult to isolate from natural sources in a pure form. It can be often isolated as chitin-glucan (from fungi) [4] and chitin-melanin from insects) [7] complexes.

Chitosan is soluble in aqueous acidic solutions due to the presence of amino-groups. The solubility is also controlled by the distribution of the acethyl groups remaining along the chain [9].

The characterization of chitin and chitosan is difficult, and numerous related studies have been carried out [9,10]. NMR, FTIR and elemental analysis only can characterize chitin in solid state due to its lack of solubility. For chitosan due to solubility more methods such as potentiometry, UV-Visible spectroscopy, viscosimetry, scanning calorimetry, scanning electron microscopy and X-ray diffraction patterns can be used. Elemental analysis can be used when chitosan is in the pure form [9-11].

Alternative raw sources of chitin, chitosan and its complexes, such as, for instance, lowest plants – mushrooms insects such as bees, colorado beetles etc. as well as industrial wastes, especially from brewing of beer and manufacture of wine and ethanol can be used for extracting chitosan-containing products. As follows from above the first and necessary stage in producing chitosan is an extraction of chitin and its complexes. The current work was done with the purpose to extract chitin and its complexes from insects (cockroaches and bees), mushrooms, banana wine and beer waste products characteristic for Rwanda and to characterize them using FTIR spectroscopy and elemental analysis.

METHODS AND MATERIALS

Pretreatment of Samples

The insects (cockroaches *Pariplaneta Americana linnaeus* and bees *Apis mellifera lineaus*) were identified in Invertebrate zoology section of the National Museum of Kenya. They were killed using chloroform, then ground. The mushrooms *Amanita phalloides* and *Lactarius subdulius* were cleaned, separated into pileus and stipes and milled. The banana wine (Kovibar and Urwibutso Inc.) and beer waste products (Bralirwa Inc., traditional *Sorghum*) were dried and ground into powder. They were the residues after yeast fermentation in wine and beer production

Extraction of Chitin and Its Complexes

A raw biomass of insects, mushrooms and dry wine (beer) waste products (150 g of each sample) was treated twice with 8% solution of NaOH at 90°C for 1 h (deproteinization process). Then demineralization was carried out with 6.7% solution of HCl at 25°C for 3h, washing from fats and lipids. After that the final product was filtered, washed out using Buchner funnel with distilled water up to neutral pH (pH~7) Finally, the product was defatted with 96% ethanol in Soxhlet's extraction apparatus for 18 h, washed out with distilled water and dried up to constant weight at 50-60°C [12].

The obtained materials must be chitin-glucan complexes (in the case of mushrooms and wine (beer) waste products) and chitin-melanin complexes (in the case of cockroaches and bees). Production of white chitin was carried out through bleaching brown chitin-melanin complexes with 33 % solution of H₂O₂ [7].

Characterization of Extracted Products Using Fourier Transform Infrared Spectroscopy (FTIR) and Elemental Analysis

The FT-IR spectra of chitin and its complexes prepared as powders were recorded on a “Spectrum One” FT-IR spectrometer (Perkin Elmer) with the Diffuse Reflectance Sampling Accessory (DRA) with 4 cm⁻¹ scale resolution. The spectra were recorded in the region of wave number between 4000 and 500 cm⁻¹. The reference FTIR spectra of chitin-glucan and chitin-melanin complexes extracted from *Aspegillus niger* mushrooms and bees material [3], respectively, were used for comparison with the spectra of the extracted products. The elemental composition of chitin and its complexes was determined using Perkin-Elmer PE 2400 CNH (USA) elemental analyzer.

RESULTS AND DISCUSSION

FTIR Spectroscopy

Identification of isolated complexes was carried out using FTIR spectroscopy (Table 1). The FTIR spectra of the chitin-glucan and chitin-melanin complexes extracted from mushrooms and bees, respectively, are given in Fig.1

TABLE 1. FT-IR vibration bands in chitin-glucan and chitin-melanin complexes extracted from the studied samples

Assignments	ChGC & ChMeC (Reference samples)	ChMeC in insects	ChGC mushrooms	ChGC in wine & beer waste products
ν OH	~3400	3420	3400 (broad absorption)	3400 (broad absorption)
ν_s NH (-NHCOCH ₃)	3265; 3100	3260; 3100	3300 (broad absorption)	3264; ~3100
ν_{as} CH(-CH ₃)	2961 w	2960 w	-	-
ν_{as} CH(-CH ₂ -)	2928 m	2925 w	2920 w	2920
ν_s CH(-CH ₂ -)	2871 m	2878 m	2887 m	-
ν_s C=O (-NHCOCH ₃)	1653 s (amide I)	1652	1642	1644
δ NH + ν CN (-NHCOCH ₃)	1558 s (amide II) 1310	1557 1305	1556 1312	1560 w -
δ_s CH (-CH ₂ -)	1376 (amide III)	1376	1375	1374
δ_{as} CH (-CH ₂ -)	1426	1426 s	1424 w	1425 w
ν_{as} C-O(-C-O-C-)	1077	1071	1070-1080 (shoulder)	1070-1080 (shoulder)
ν_s C-O(-C-O-C-)	1024	1025	1028	1025
Quinone, pyrrole, thiopyrrole	1628 s	1622 s	-	-
C=O, COOH	1710 w	-	-	1710
Heterocycles	1420 w	~1420 w	~1420 w	~1420 w
Dihydroxi-5,6-indor polymer	1220	1215 w	-	-
	1157(2 peaks with similar height)	1160 w	-	-

There were found the similarities for FTIR spectra of chitin-glucan (ChGCs) and chitin-melanin complexes (ChMeCs) extracted from different natural sources (Fig.1).

The presence of chitin parts causes the absorption band at 1642¹ (1652)², 1555¹ (1557)² and 1375¹ (1376)² cm⁻¹ and in the vicinity of 3260² and 3099² cm⁻¹, which correspond to vibrations of amide groups «amide I», «amide II» and «amide III», respectively (¹ & ² correspond to absorption bands of chitin component of ChGCs and ChMeCs, respectively). For reference chitin-glucan and chitin-melanin complexes the values of the same absorption bands are 1653, 1558 and 1376 cm⁻¹ and in vicinity of 3265 and 3100 cm⁻¹. The presence of absorption bands in the 3400-2800 and 1160-1020 cm⁻¹ in the spectra of the studied samples indicates the existence of glucan and melanin parts. These absorption bands appear due to N-H, O-H, C-H and C-O, C-C vibrations which manifest in the spectra both chitin and glucan. Melanin has also the characteristic absorption bands at 1621(1622) cm⁻¹ of significant intensity and corresponds to ring structures typical for melanin involved pyrrone, γ -thiopyrrone, indole, and quinone [3,13].

The character of the chitin-glucan bonds in the ChGCs remains disputable. According to the published data [13] the ChGC is a complex system, in which chitin is bonded to different glucan forms. If chitin is the predominant component (*Amanita phalloides* mushrooms, Table 1), it serves as a backbone, while glucan forms side chains; in contrast, glucan composes the backbone and chitin forms side chains when glucan prevails (*Lactarius subdulius* mushrooms, wine (beer) waste products).

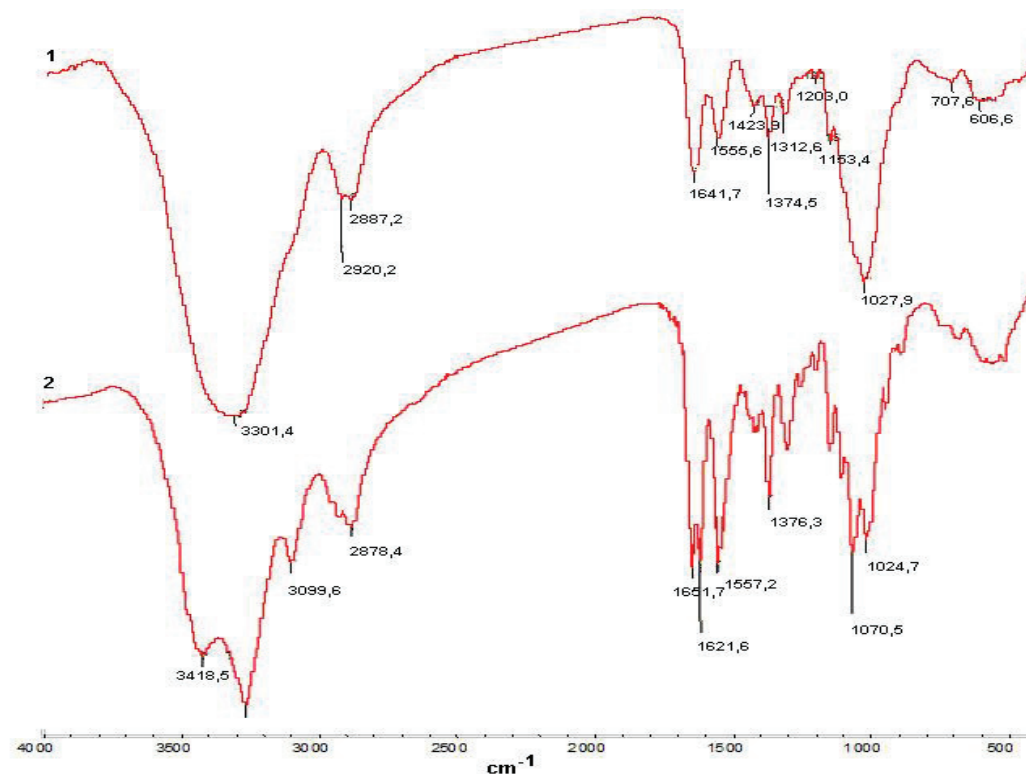


FIGURE 1. FTIR spectra of chitin-glucan extracted from *Lactarius subdulius* mushrooms (1) and chitin-melanin complexes extracted from bees *Apis mellifera lineaus* (2)

The chemical composition of the raw sources of ChGC studied and the chitin-to-glucan ratio vary with the types of raw product and with some special conditions (soil, climatic, ecological and cultivation) of growing (for mushrooms) and production (wine (beer) wastes).

As follows from the Table 1, there are different ChGCs in mushrooms and wine (beer) waste products as indicated by a non-identity of their FTIR spectra. The chitin symmetric stretching (ν_s CH(-CH₂-) at 2887 cm⁻¹) and bending and stretching (δ NH + ν CN(-NHCOCH₃) of amide II at 1310 cm⁻¹) vibrations are missing from the spectrum of ChGCs of wine (beer) wastes.

Elemental Analysis

The elemental composition of chitin and its complexes was determined by elemental analysis. The percentage of C, N, H elements was determined as followed in Table 2.

In general, the structure of the ChGC may be depicted by the formula :

$$[C_8H_{13}O_5N]_x[C_6H_{10}O_5N]_y[H_2O]_z, \quad (1)$$

where $[C_8H_{13}O_5N]$ and $[C_6H_{10}O_5N]$ are the glucan and chitin components of a ChGC, respectively; x, y, z are the fractions of the glucan, chitin and water parts, respectively

The structure of the chitin-melanin complexes (ChMeCs) may be depicted by the formula :

$$[C_{77}H_{98}O_{33}N_{14}S]_x[C_8H_{13}O_5N]_y[H_2O]_z, \quad (2)$$

where $[C_8H_{13}O_5N]$ and $[C_{77}H_{98}O_{33}N_{14}S]$ are the melanin and chitin components of a ChGC, respectively; x, y, z are the fractions of the melanin, chitin and water parts, respectively.

The formulas (3)-(6) below were used for calculation of the fractions of chitin, glucan, melanin components and H₂O in all the extracted chitin-containing samples They are as follows:

$$\%C_{total} = (\%C_{in\ glucan\ or\ melanin\ component}) \times x + (\%C_{in\ chitin\ component}) \times y \quad (3)$$

$$\%H_{total} = (\%C_{in\ glucan\ or\ melanin\ component}) \times x + (\%H_{in\ chitin\ component}) \times y + (\%H_{in\ H_2O}) \times z \quad (4)$$

$$\%N_{total}(for\ ChGC) = (\%N_{in\ chitin\ component}) \times y \quad (5)$$

$$\%N_{total}(for\ ChMeC) = (\%N_{in\ melanin\ component}) \times x + (\%H_{in\ chitin\ component}) \times y \quad (6)$$

A percentage of the main elements C, H, N in chitin, glucan and melanin components of chitin-containing complexes given in formulas (3)-(6) was determined as a ratio of the molar mass of the main elements in the above-mentioned parts in general formulas and the molar mass of these parts.

TABLE 2. Percentage of the main elements in chitin-glucan and chitin-melanin complexes extracted from mushrooms, insects and wine and beer waste products and their composition

Sample	Percentage, %			Formula
	C	H	N	
Cockroaches <i>Pariplaneta Americana linnaeus</i>	47.23	7.32	7.20	(C ₇₇ H ₉₈ O ₃₃ N ₁₄ S) _{0.09} (C ₈ H ₁₃ O ₅ N) _{0.9} (H ₂ O) _{0.002}
Bees <i>Apis mellifera linnaeus</i>	52.65	8.42	5.55	(C ₇₇ H ₉₈ O ₃₃ N ₁₄ S) _{0.03} (C ₈ H ₁₃ O ₅ N) _{0.8} (H ₂ O) _{0.004}
Mushrooms				
<i>Amanita phalloides</i> (brown)	43.16	7.41	3.52	(C ₆ H ₁₀ O ₅) _{0.43} (C ₈ H ₁₃ O ₅ N) _{0.51} (H ₂ O) _{0.14}
<i>Lactarius subdulius</i> (white)	42.83	7.38	1.75	(C ₆ H ₁₀ O ₅) _{0.69} (C ₈ H ₁₃ O ₅ N) _{0.25} (H ₂ O) _{0.13}
Wine waste products				
Kovibar Inc.	41.38	6.54	0.36	(C ₆ H ₁₀ O ₅) _{0.88} (C ₈ H ₁₃ O ₅ N) _{0.05} (H ₂ O) _{0.07}
Urwibutso Inc.	40.87	6.42	0.47	(C ₆ H ₁₀ O ₅) _{0.82} (C ₈ H ₁₃ O ₅ N) _{0.07} (H ₂ O) _{0.08}
Beer waste products				
Bralirwa Inc.	41.87	6.16	0.88	(C ₆ H ₁₀ O ₅) _{0.81} (C ₈ H ₁₃ O ₅ N) _{0.13} (H ₂ O) _{0.03}
Traditional Sorghum	44.65	6.30	1.50	(C ₆ H ₁₀ O ₅) _{0.77} (C ₈ H ₁₃ O ₅ N) _{0.22} (H ₂ O) _{0.01}

The ratios of chitin, glucan and melanin components presented in Table 2 allowed to estimate a percentage of chitin in all the samples extracted (Table 3) using the following proportion :

$$\% \text{ chitin} = (y \times 100\%) / (x + y + z) \quad (7)$$

TABLE 3. Yield and chitin composition of the extracted products

Raw source	Yield , %	% of chitin composition (to dry mass (DM) of a raw material)
Cockroaches <i>Pariplaneta Americana linnaeus</i>	42	38
Bees <i>Apis mellifera lineaus</i>	23	22
<i>Amanita phalloides</i> mushrooms	11.1	5.2
<i>Lactarius subdulius</i> mushrooms	11.7	2.5
Wine wastes of Kovibar Inc.	13	0.7
Wine wastes of Urwibutso Inc.	11.3	0.8
Beer wastes of Bralirwa Inc.	5.33	0.7
Traditional Sorghum Beer wastes	17.6	3.9

From Table 2 we notice that for raw sources of chitin used in this study a fraction of chitin component was found to be greater than that of glucan and melanin components. According to Table 3, the lowest amount of chitin was extracted from wine and beer wastes, whereas the highest chitin quantity was obtained from cockroaches

Pariplaneta Americana linnaeus and bees *Apis mellifera lineaus*. These results, in particular, in respects of insects and mushrooms correlate well with literature data for other species [14].

SUMMARY

Chitin –glucan and chitin-melanin complexes were successfully extracted from natural raw sources such as insects (cockroaches *Pariplaneta Americana linnaeus* and bees *Apis mellifera lineaus*), mushrooms (*Amanita phalloides* and *Lactarius subdulius*), waste banana wine (Kovibar and Urwibutso Inc.) and beer products (Bralirwa Inc., traditional sorghum) characteristic for Rwanda using standard procedures of deproteinization and demineralization with the yield ranged between 5.33 % in the case of Beer wastes of Bralirwa Inc. and 42% characteristic of cockroaches. They have been identified using FTIR spectrophotometry comparing with the reference samples of *Aspegillus niger* mushrooms (for chitin-glucan complexes) and bees material (for chitin-melanin complexes).

The elemental composition of chitin and its complexes was determined by elemental analysis. For insects *Pariplaneta Americana linnaeus*, *Apis mellifera lineaus* and *Amanita phalloides (brown)* mushrooms a fraction of chitin part were found to be greater than that for melanin (glucan) components. The chitin content of the samples studied ranged between 0.7 % of DM (dried mass of raw material) for wine (beer) waste products and 38% of DM for cockroaches.

The chitin extracted from cockroaches is thus the most suitable for chitosan production. The large numbers of cockroaches captured provide an abundant source for the production of chitin. In addition, attempts to breed them will help to make such unpleasant insects useful for people.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research (Grant 14-03-00898), the Program 211 of the Government of the Russian Federation № 02.A03.21.0006 and the State Tasks of the Ministry of Education (Russian Federation) No. 4.1626.2014/K and No. 2014/239 and the local Grant of University of Rwanda “Chitosan-containing materials of multifunctional application for needs of Rwanda”. The authors are thankful to J.P. Intwali (Rwanda), E.V. Habumugisha (Rwanda), Ch. Ukundineza (Rwanda) and D. Niyoyita (Rwanda) for capturing insects and gathering mushrooms and their initial preparation for chitin extraction.

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